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Presence of Glyphosate in First Order Streams Associated with Rainfall Events

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Abstract

Glyphosate is widely used in the United States and has recently been described as both mobile and persistent in soils and bodies of water. There is little research however, on the variability of glyphosate concentration in runoff based on different land use types. For this study an evaluation of samples from different land uses are used to assess glyphosate concentrations in first order streams during a runoff event. The intent was to compare three sites with a. known commercial applications, b. known lack of applications (organic), and c. assumed limited application of glyphosate. There was no significant difference in glyphosate concentration in first order streams between these locations. There was a small positive correlation between stream depth and glyphosate concentration at the c. location. There were more dissolved solids at the b. location when compared to the other two locations. Collectively the data suggests there is no statistically significant variability in glyphosate concentrations at these three sampling locations. While detected concentrations are low, these findings indicate a potential trend of overall glyphosate persistence in the environment and the persistence of glyphosate in runoff into bodies of water.

Introduction

History:

Glyphosate is a nonselective herbicide sold as the weed killer Roundup® and is effective through inhibiting enzyme activity in plants (Dragus, 2015). It has been used worldwide for over 40 years and became popular with the introduction of transgenic Roundup Ready® soybeans (*Glycine max),* corn (*Zea mays)* and cotton (*Gossypium spp.)* (Rubio, 2003). Since the expiration of the Roundup ® patent, glyphosate of all forms became accessible at neighborhood stores that sell planting materials such as Lowe's ® and The Home Depot ®. Glyphosate has been regulated by the Safe Drinking Water Act since 1986 and its maximum contaminant level is 0.7 mg/L (700 ppb) (EPA, 2009). Glyphosate has been continuously reviewed and deemed safe and effective to use as a weed killer because it was observed to photodegrade. However, glyphosate usage in the US alone increased from about 5,000 to 80,000 metric tons/year between 1987 and 2007 (Battaglin, 2014). This increase in concentration and usage has led to the description of glyphosate as both mobile and persistent. Initial testing by Monsanto Chemical Co. labeled glyphosate with a half-life of 20 days (Ermakova, 2010). While glyphosate degradation can occur in a variety of ways, including adsorption, photocatalytic degradation, and microbial degradation, there is little review of degradation of glyphosate in the environment at current levels. This creates a paradox of understanding as this is one of the most used herbicides in the world and yet little is known about its environmental fate. Use of this herbicide has been approved for a variety of settings including agricultural and nonagricultural areas. Over 100 use locations have been approved for glyphosate including roads, canals, around public spaces and homes (Perry, 2019). This explains the appearance of this chemical in multiple locations around the US and potentially explains recent court settlements that recognize Roundup® as a source of cancer (Battaglin, 2014).

Health Risks:

Human

In 2015 the International Agency for Research on Cancer listed glyphosate as a potential carcinogen with a potential link to non-Hodgkin lymphoma (NHL) (Andreotti, 2018). While this research did not show statistically significant links between cancer and site application, there was an increased risk of acute myeloid leukemia (AML) in users that were exposed to the above median range of 8.5 years of usage. In this study 7290 incidents of cancer were reported. In 2018, 36 samples from 200 stored samples of Wisconsin farmers' urine were tested, and of this 18 were from farmers that reported applying glyphosate and 17 were from known non-glyphosate users (Perry, 2019). 39% of the applicators who used glyphosate were found to have glyphosate in their urine samples whereas the non-glyphosate applicators were found to have no measurable detection of glyphosate in their urine samples. The highest detection found was 12 ppb. A risk assessment study found that exposure as little as 20 minutes of direct inhalation contact can cause damage to epithelial cells and mitochondrial functions and can cause micronuclei increase release in humans (Koller, 2012) There is also the potential for human hormone function disruption, endocrine disruption, inhibition of transcription in estrogen factors and persistence in the placenta (Gill, 2018). All of these combined effects are being further researched as more chronic exposure cases are being brought to court. Immediate exposure can cause damage, but the chronic exposure is what research indicates is leading to cancer (Andreotti, 2018).

Animal

Glyphosate toxicity assessments have been conducted for a variety of organisms including unicellular organisms, fungi, microbes, multicellular algae, invertebrates, and fish (Gill, 2018). There are many recorded confirmations of the deleterious impacts of glyphosate on aquatic and terrestrial species (Dragus, 2015). Exposure time as little as one hour at 10 mg/L had detrimental effects on bees and their ability to recall flight paths from the hive (Balbuena, 2015). Exposure time as little as 24 hours at 3-3000 mg/L had adverse effects on the ability of the amphibian species *Leptodactylus latrans* (frog) to swim and caused oral abnormalities as well (Bach, 2016). Unchecked overuse of glyphosate is causing these threats to multiple species of unicellular and multicellular organisms and its persistence is only increasing its toxicity in the environment. Not only are soil species being directly affected, but aquatic species are now considered for toxicity as glyphosate is appearing in streams in rivers around the United States.

Environmental Risk:

As glyphosate is one of the many herbicides to be present in the environment, more research is being done to understand its role in the environment long term. This compound has been found to be extremely motile and soluble in water. USGS and USEPA worked together in 2014 to publish a document in which 3,732 water samples from across the US were collected and tested for glyphosate and its byproduct aminomethlyphosphonic acid (AMPA) (Battaglin, 2014). In about 40% of all the samples glyphosate was detected and about 55% of all the samples AMPA was detected. This confirmed scientists’ belief that this substance was both motile and soluble in water. With all the contributors to glyphosate in the environment, industrial farming and household application, it is reasonable to assume that levels in the environment are increasing.

More recent research in Argentina is beginning to look at the persistence of glyphosate in surface soils and also in rainwater in agricultural areas. In fields with commercial spray application of glyphosate, glyphosate was detected in 52% of rainwater samples and in up to a meter into the soil profile of the location (Lupi, 2019). In the experimental setting, glyphosate was found to retain up to 88% in the surface soil layer. This experiment also looked into the effect of spray drift during application and leaching. These contributions were considered important when assessing glyphosate aerial mobilization and subsequent retention in rainwater.

Urban uses of glyphosate are now being considered as contributing to appearance in stream water in non-agricultural areas. In 40 samples taken from 10 states across the US, downstream from a wastewater treatment plant (WWTP), glyphosate and it byproduct (AMPA) were present in 67.5% of samples (Koplin, 2006). This correlation shows that not only is it running off into streams, but it is not being removed in WWTPs. This research again supports the suggestion that glyphosate and its byproduct are both extremely soluble and mobile in aquatic systems.

Research:

The goal of this research is to determine if glyphosate is appearing in measurable levels in streams at variable concentrations due to the runoff from limited application areas, farms that commercially apply Roundup® and other herbicides, and farms that do not apply Roundup® or other herbicides. While the presence of glyphosate has been widely confirmed, there is little to no distinction of concentration based on land use beside the obvious appearance in commercial agricultural runoff and application. It is assumed that runoff from industrial farming should have the highest levels, but with the availability of products like Roundup®, and other glyphosate containing herbicides, to the public there is a possibility that suburban runoff is contributing more to the presence of this substance in streams. It is hypothesized that glyphosate will appear in the highest levels in streams that receive suburban runoff, and in the lowest levels in streams that receive runoff from herbicide-free (noncertified organic) farmland.

Methods

Collection Site:

Three locations for sampling of glyphosate were selected. These locations were all in central Ohio, all three had a first order stream with runoff contributions from surrounding areas. From the USGS web soil survey, each location sampled had a hydrologic soil group rating below A and mainly C and D, which is overall low infiltration and high runoff potential when thoroughly wetted. These locations were mainly silt loam soils or silty loam soils with little clay. Each location was selected because of the type of land usage classified adjacent to or surrounding the stream for sampling. This research was intended to observe first order streams specifically so that runoff attribution would be influencing what was appearing in the streams during a rainfall event. A first order stream is classified as a tributary to other streams and does not have water flowing through it at all points of the year. The first site was a first order stream off a suburban development. This public site was County Line Run in Westerville, OH with coordinates 40.1318126227937, -82.9405520348495. This was assumed to be an intermediate site with noncommercial application in the runoff; however, there would still be exposure to herbicides from local application by homeowners. There is potential for lawn company commercial application but theoretically this is local application on driveways and sidewalks not on the same scale as large agricultural application. This site was sampled from 6/2/2019 until 6/9/2019.

The second site selected was a noncertified organic farm in Delaware, OH (as a condition of sampling, the privacy of the owner was agreed to and location specifics are withheld). I was granted permission to take samples from this location by the owner of the property. This site was selected because it is a noncertified organic farm and in speaking with the owner there is known lack of herbicide application altogether. They have rotational crop planting of corn and wheat with pasture for their small herd of cattle, sheep, and goats. The stream for sampling was directly on the property adjacent to their fields which ideally limited glyphosate contributions from local residential homes in the surrounding area of this location. This site was sampled from 6/24/2019 until 6/28/2019.

The final site sampled was an agricultural property in Sunbury, OH (as a condition of sampling, the privacy of the owner was agreed to and location specifics are withheld) that has been farmed for approximately 25 years and has recorded application of glyphosate on the property. This information comes from the real estate agent during the recent property purchase which included a comprehensive list of all recorded chemical application on site. I had permission from the new owner to obtain this information and to sample this location on their property. It is important to note there was commercial site glyphosate application in the years prior to this sampling, but not the year of sampling due to the new purchase. The stream was in the woods directly adjacent to the commercially applied farmland. Other large farm properties were in the area, but it is unknown whether there was commercial application on those lands. This site was sampled from 8/18/2019 until 8/24/2019.

Sample collection was planned around rainfall events that would be sufficient enough to generate runoff, and the season was selected due to typical seasonal planting patterns. Summer planting seasons are typical for corn and soybeans. Due to entering onto private properties, sampling had to be spread out throughout the whole summer. Prior to installation, I checked the weather for the week to determine if it was going to rain enough to produce measurable runoff, based on weather projections. It was key to have rainfall during the sampling period as to compare the runoff between the three locations rather than typical stream levels.

Sampling Procedure:

Samples were taken from each of the three locations over a period in which there was a rainfall event that increased runoff. Some samples have the rainfall event occurring towards the end of sampling period and others happen towards the beginning. During the sampling week, each location had an ISCO water sampler and *In-Situ* Aqua TROLL 200 datalogger installed.

Figure 1: From left to right- Installation of the ISCO water sampler at suburban location, organic location, and commercial exposure location.

Water samples were taken every twelve hours over the course of the sampling period as set by the ISCO autosampler pre-programmed for each location. Each water sample taken at the 12-hour mark was 800 ml, but only 100 mL were frozen for sampling. During that sampling period, the *In-Situ* Aqua TROLL 200 datalogger was also installed at the location taking real time data of: temperature (F), depth (ft), resistivity (ohm-cm) and total dissolved solids (ppt) at concurring intervals with the ISCO sampler. These were the units set as the standard on the device. At the end of the sampling period all the equipment was collected and returned to the lab where the data was analyzed. Water samples were frozen in plastic test tubes with lids in accordance with the

Abraxis storage recommendation for long term ELISA samples. Data was downloaded from the *In-Situ* Aqua TROLL 200 datalogger into the Win-Situ 5® application in which historical interval data from the week could be compiled into hydrographs.

Detection of Glyphosate:

After all samples were collected, they were run through a glyphosate ELISA kit Assay from Abraxis Bioscience, Product No. 500086. Procedures were followed as ascribed in Abraxis Glyphosate ELISA kit procedures. Samples were thawed and then prepped with the standards and controls from the kit and deposited into the microtiter plate via an 8-channel multichannel pipette. Glyphosate was recognized on the plate by polyclonal antibodies. The intensity of the color from the substrate solution was inversely proportional to the concentration of glyphosate. The microtiter plate was then run through the plate reader which determines concentration from an absorbance reading at 450nm. Concentration is determined using the standard curve run with each test. Detection limit is 0.05 parts per billion (ppb) and mean detection limit is 0.5ppb. Results were evaluated by calculating the mean absorbance value for each standard and constructing a standard curve, which was done automatically through the microtiter plate reader computer program. Again, all followed procedures and result analysis is described in the purchased ELISA kit. This method was selected as most of the inorganic and organic substances found in water samples have been tested in up to 10,000 ppm and found not to influence glyphosate results.

The ELISA method is as effective as the high-pressure liquid chromatographic (HPLC) method with a lower limit of detection (Rubio, 2003). It is also known that the river matrix has little to no effect on the ELISA results compared to the HPLC method as well. The ELISA is a viable, and becoming more common, method for glyphosate detection in water samples and can measure water quality guidelines for both North America and Europe.

Statistical Analysis:

Parts per billion glyphosate was analyzed as a completely random design with 9 to 14 replication depending on location. Student-Newman-Keuls (α=0.05) tests were used to determine statistical significance between means for locations. Data was compiled into excel spreadsheets including ppb glyphosate and Win-Situ 5® data to build graphs for comparison. Averages and standard deviations were calculated for the *In-Situ* Aqua TROLL 200 data.

Results

Relative glyphosate levels were low at all three locations with no significant difference between them. At the noncommercial limited exposure location average glyphosate was 0.20 ppb. At the organic location average glyphosate was 0.22 ppb, and at the commercial exposure location average glyphosate was 0.25 ppb (Figures 2, 4, 6 respectively). There were glyphosate fluctuations throughout the sampling period with the largest fluctuations occurring at the noncommercial limited exposure location (Figure 2).

There were fluctuations in depth, total dissolved solids and resistivity at each location throughout the duration of sampling (Figures 3, 5, 7). These fluctuations indicate changes in the physical characteristics of each location. Depth fluctuations indicate runoff is occurring from rainfall events causing the overall rise in stream depth. Total dissolved solids indicate particulate matter fluctuations in the stream also indicative of turbulence or runoff in the stream. Resistivity indicates dissolved salts; low resistivity indicates high dissolved salt concentrations. In total dissolved solids, the organic location yielded a significantly higher number of dissolved solids compared to the commercial exposure location and the noncommercial limited exposure location (Figure 10). Comparisons in depth and resistivity were not considered significant.

There appears to be little to no correlation between the dissolved solids and the glyphosate concentration; however, there seems to be a slight correlation between glyphosate concentration and water level (Figure 8). This is a positive correlation but due to the small R^2 value this cannot be deemed entirely linear (Figure 9). The only location where this correlation was notable was the noncommercial limited exposure location.

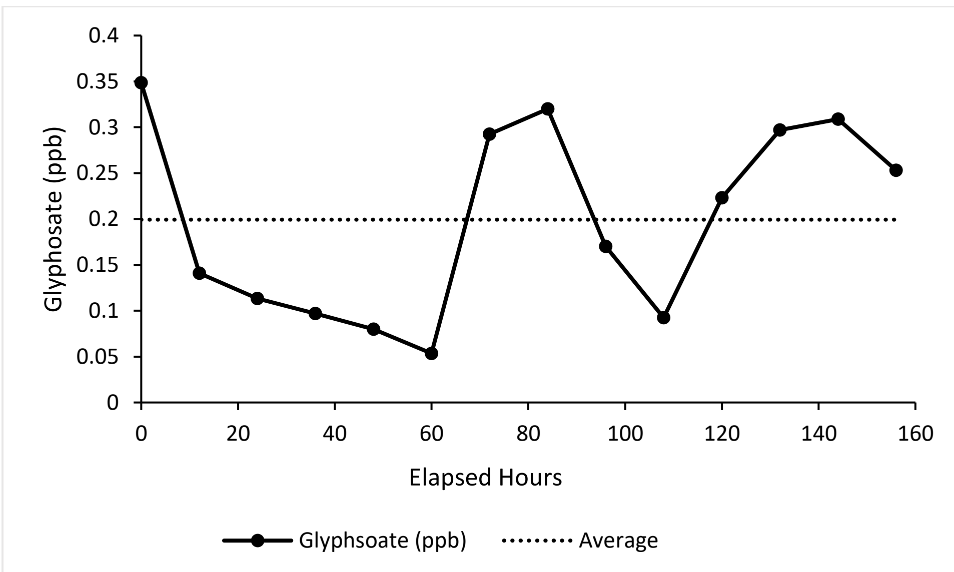

Figure 2: Assumed noncommercial limited exposure location (suburban): Glyphosate in parts per billion every 12 hours over the course of 156 hours of sampling, with the overall average amount from that location shown as a dotted line.

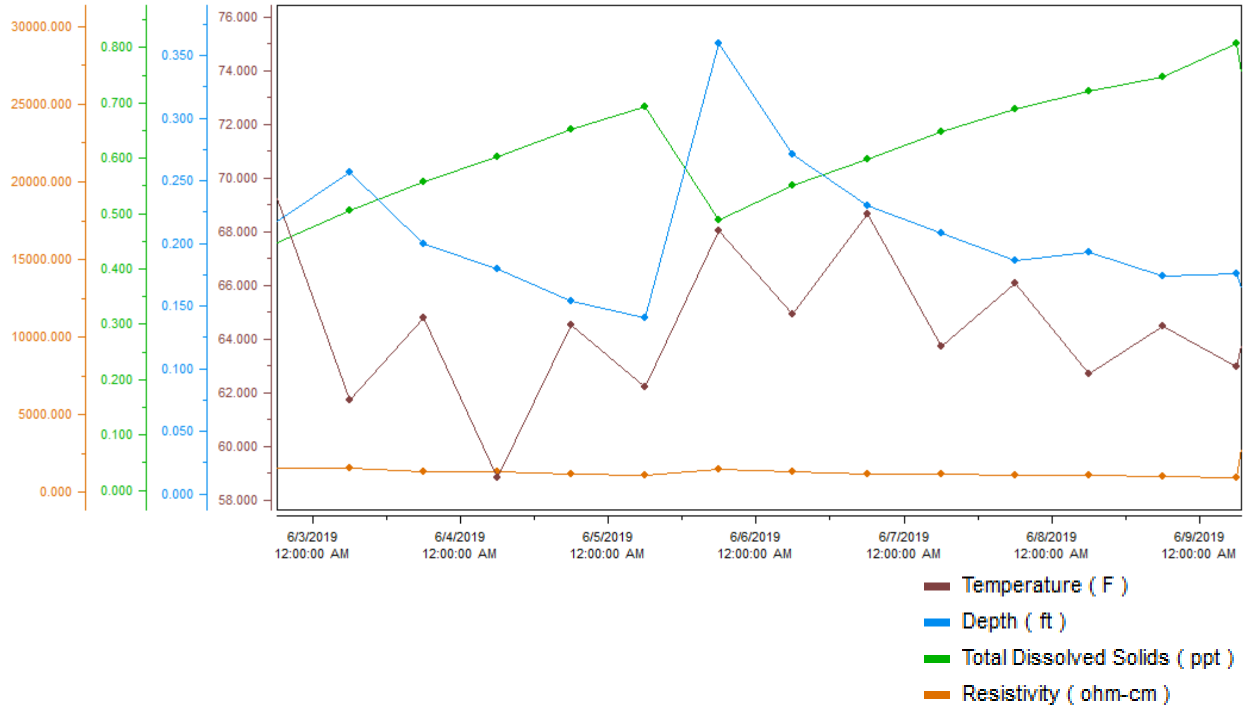

Figure 3: Assumed noncommercial limited exposure location (suburban): Temperature in Fahrenheit, Depth in feet, Total Dissolved Solids in parts per trillion, and Resistivity in ohm-cm measured every 12 hours over the course of 156 hours of sampling during a rainfall event.

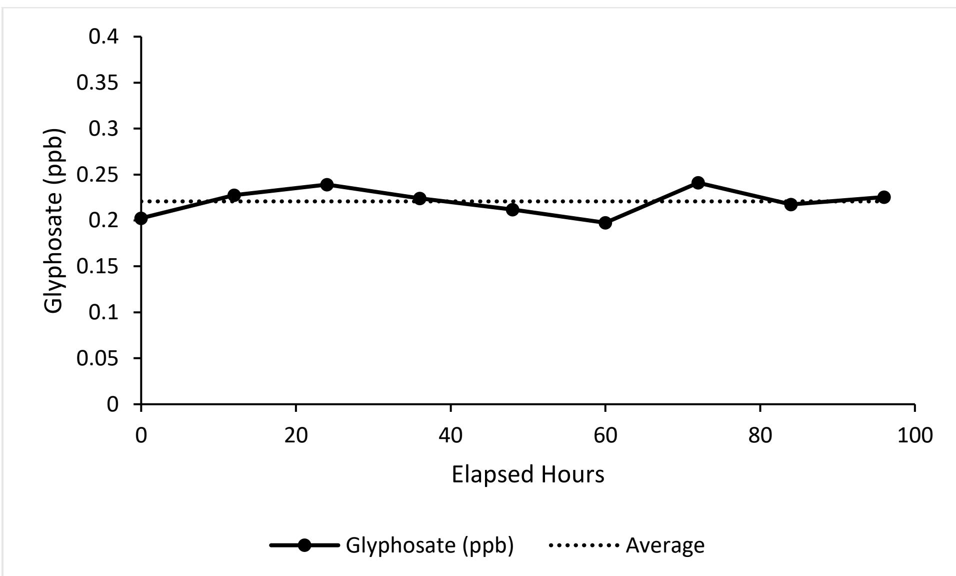

Figure 4: Assumed no exposure (organic) location: Glyphosate in parts per billion every 12 hours over the course of 96 hours of sampling, with the location average shown as a dotted line during a rainfall event.

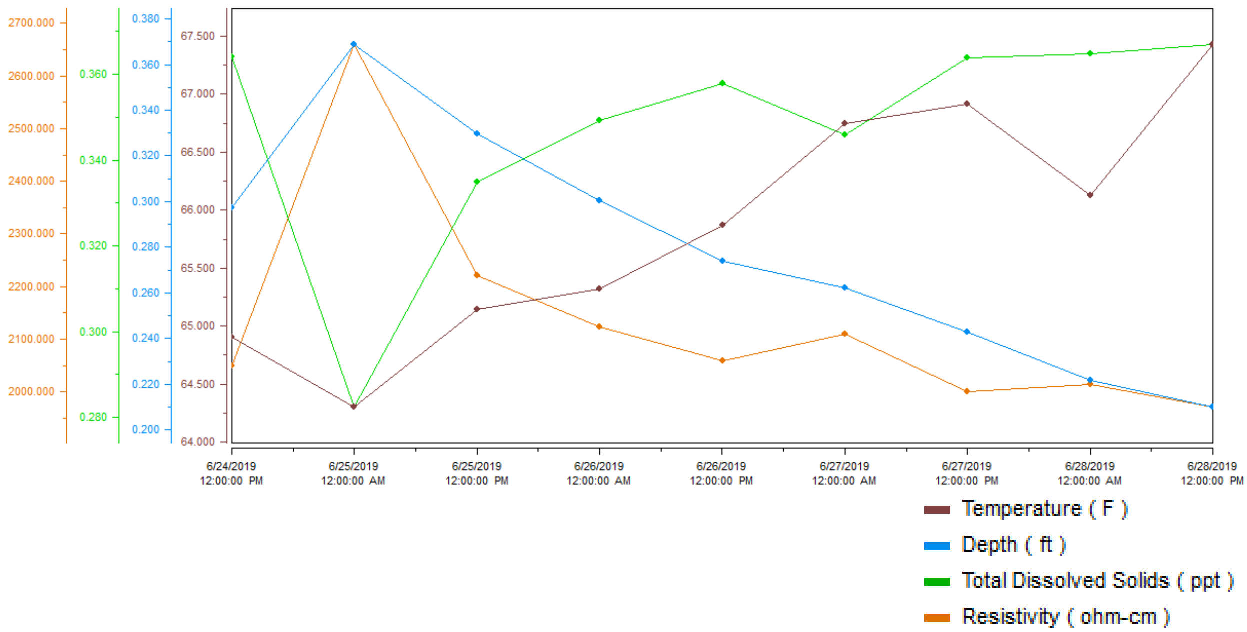

Figure 5: Assumed no exposure (organic) location: Temperature in Fahrenheit, Depth in feet, Total Dissolved Solids in parts per trillion, and Resistivity in ohm-cm measured every 12 hours over the course of 96 hours of sampling during a rainfall event.

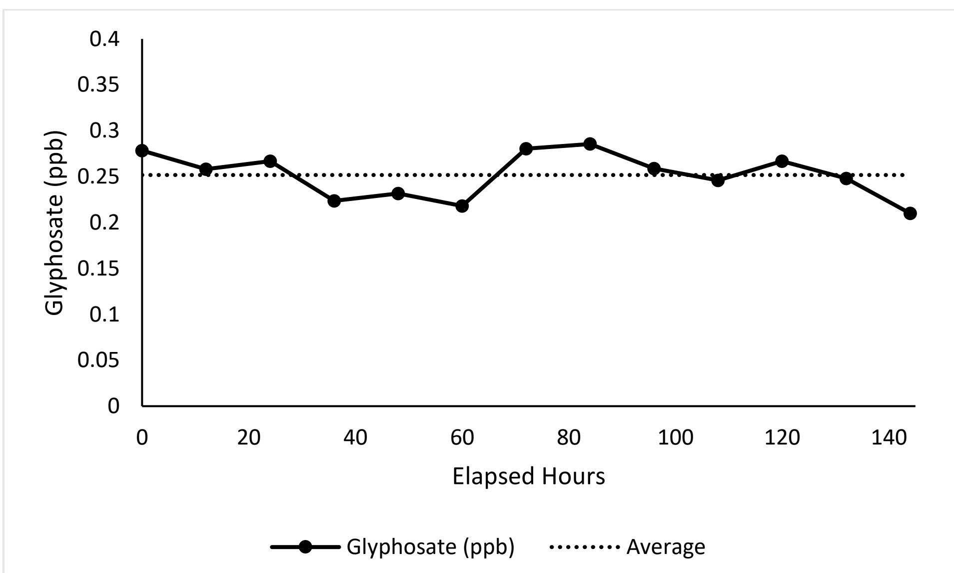

Figure 6: Known historical commercial application location: Glyphosate in parts per billion every 12 hours over the course of 144 hours of sampling, with locational average shown as a dotted line during a rainfall event.

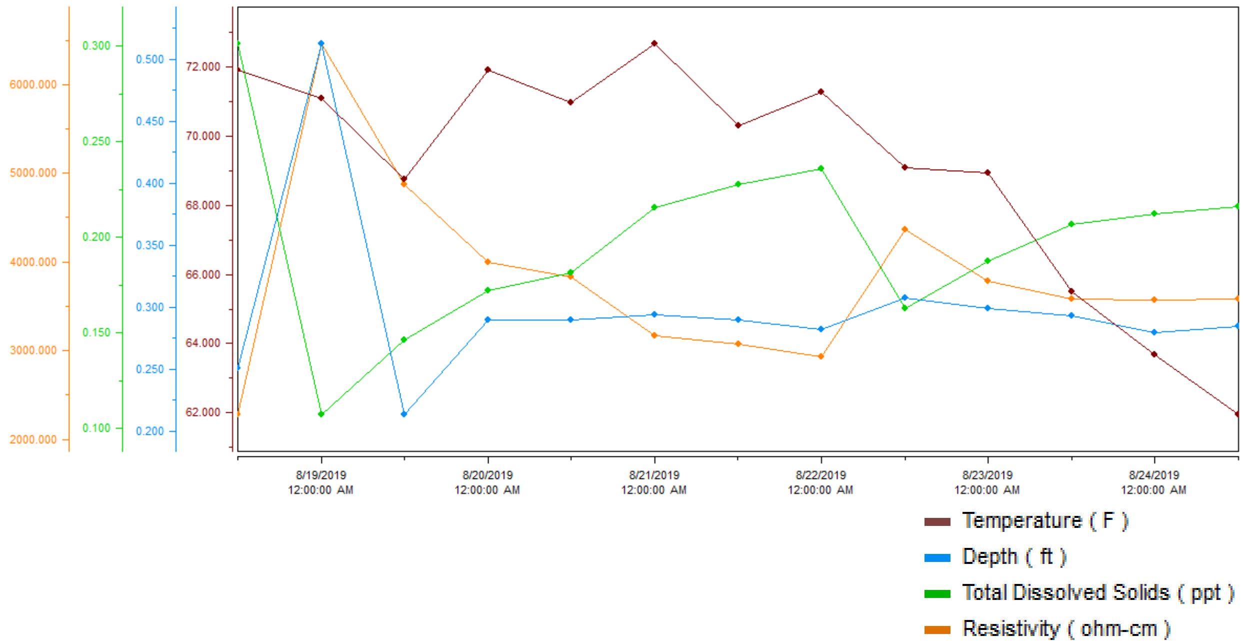

Figure 7: Known historical commercial application location: Temperature in Fahrenheit, Depth in feet, Total Dissolved Solids in parts per trillion, and Resistivity in ohm-cm measured every 12 hours over the course of 144 hours of sampling during a rainfall event.

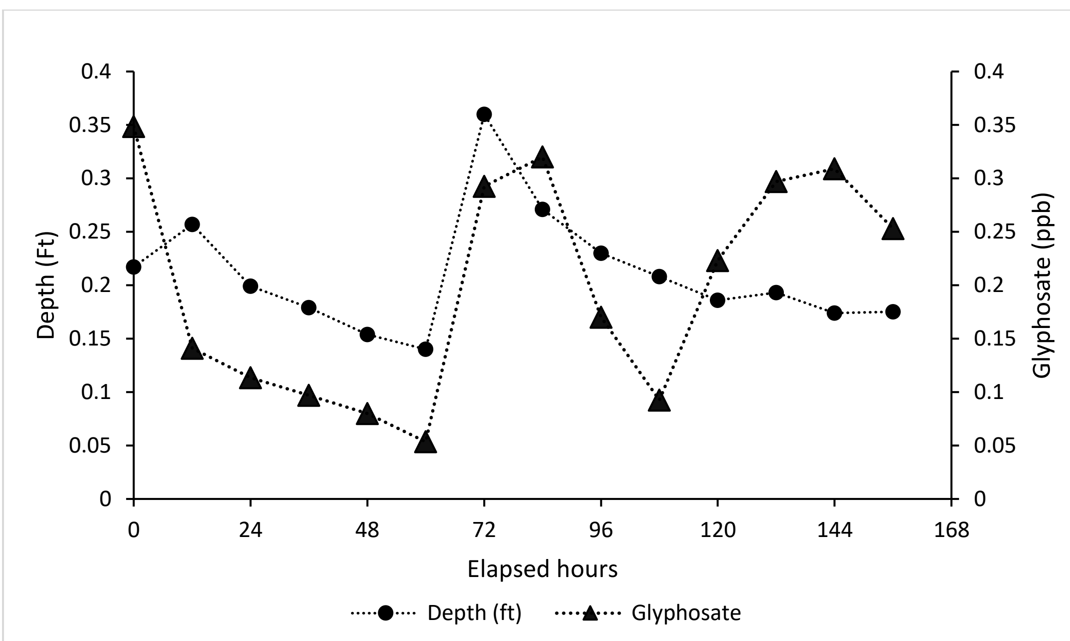

Figure 8: Assumed noncommercial limited exposure location (suburban): Glyphosate measured in ppb and depth measured in feet taken in 12-hour intervals over the course of 156 hours.

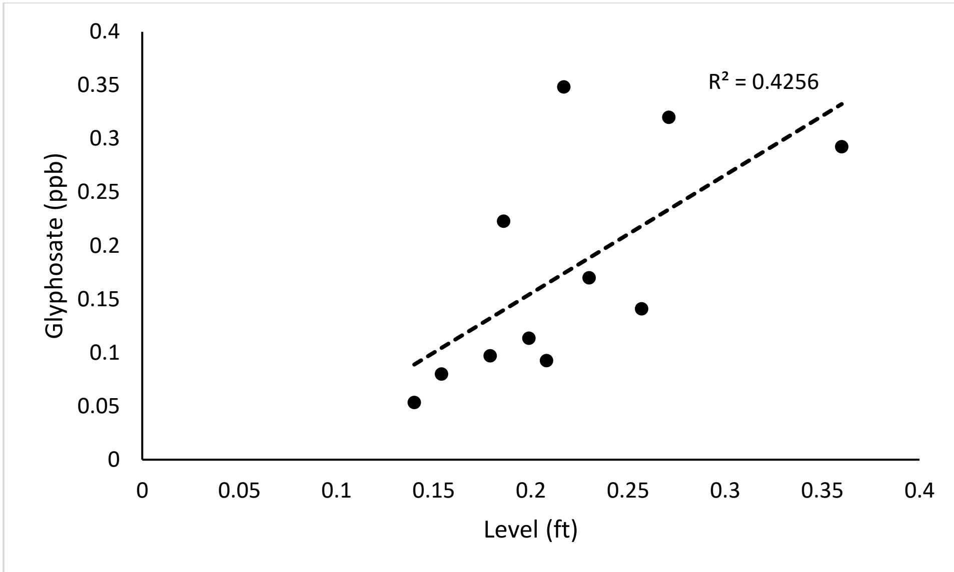

Figure 9: Assumed noncommercial limited exposure location (suburban): Glyphosate measured in ppb and depth measured in feet compared at 12-hour intervals over the 156 hours of data collection.

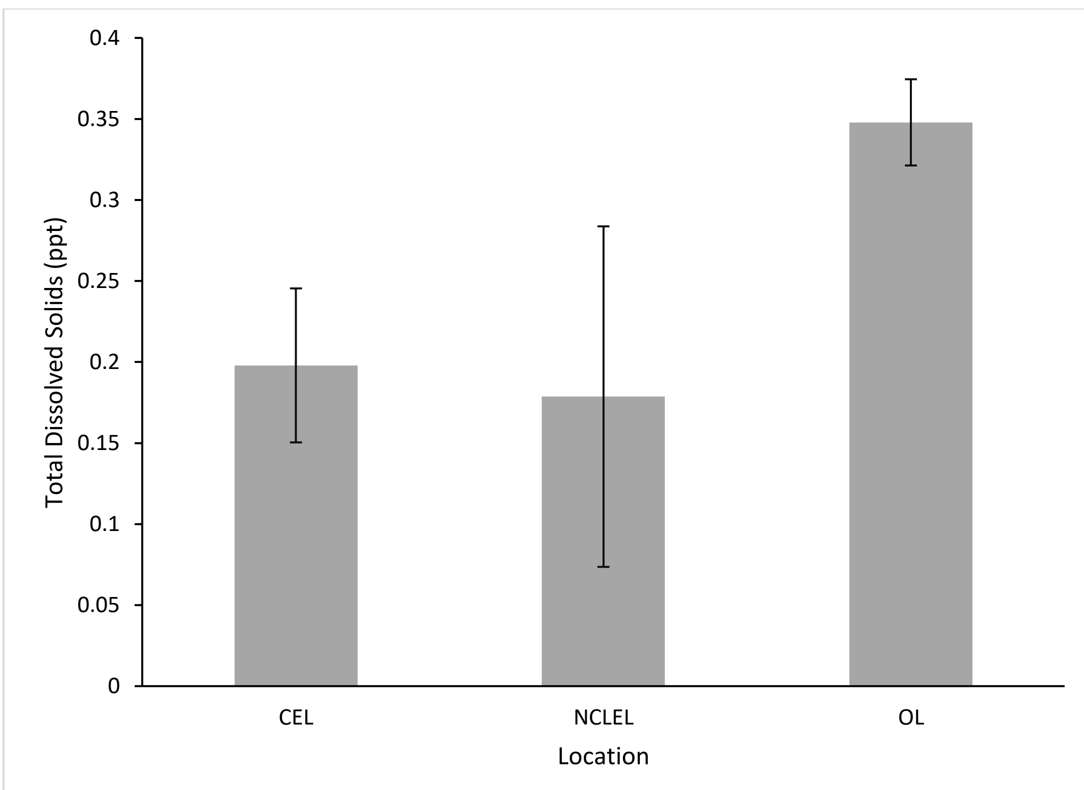

Figure 10: Average total dissolved solids in parts per trillion at the three locations. The bars represent the average and standard deviation of the samples.

Discussion

In 2014 USGS and USEPA found glyphosate in 71.6% of stream samples from 1,508 samples from around the United States including Ohio (Battaglin, 2014). From all these stream samples median glyphosate level was 0.03 ppb. There was also a median glyphosate detection of 9.6 ppb in the soils and sediments (from 45 samples) and 0.11 ppb in precipitation (from 85 samples). The average glyphosate level detected in this research was 0.22 ppb and it was found in 100% of samples. Average glyphosate from this research is approximately seven times higher than what was observed in streams in 2014, and this increase occurred in just five years. Median glyphosate in ditches in drains from the 2014 survey was 0.2 ppb which is closer to the average that was observed in this research. While this research did not look at soil concentrations and precipitation concentrations, previous research indicates soil and precipitation contributions to glyphosate in streams. This may further support the glyphosate runoff and persistence theory proposed initially. If glyphosate is remaining in sediments and concentrating at those ditch and drain locations, then it would make sense that it is leaching into runoff and being moved. If glyphosate is persisting in soils, sediments, and precipitation, then those factors would more than likely be contributing to glyphosate appearance in streams.

This research was intending to correlate the types of land use to observed glyphosate concentrations, but instead showed that there is little to no difference between these three locations. This may indicate that there is a base level of glyphosate that is expected to be present in stream water due to the increased agriculture that exists in Ohio and increased availability of glyphosate to individual homeowners. If this is the case in Ohio, then there would be expected minimum levels observed throughout stream sampling, and this could be reflected in the similarities between the three sampling sites. Other research supports the motility and mobility of glyphosate and its byproduct AMPA as well. In Argentina researchers discovered that there is leaching and runoff of glyphosate, and in rainwater glyphosate and its byproduct were found in over 52% of samples (Lupi, 2019). Kolpin et al. found glyphosate and its degradate in 67.5% of 40 samples taken throughout the US in streams and in cases specifically downstream of wastewater treatment plant (WWTP) effluent samples. The persistence of glyphosate may indicate a general increase in the concentration in stream water samples in Ohio and may prove that overall concentrations in the environment are increasing. The 0.03 ppb to 0.2 ppb increase occurred over the course of 5 years and may indicate a trend of increasing levels as usage continues to persist. Clair Patterson described anthropogenic global pollution through measuring lead in sediment in the ocean. Patterson's work discovered that lead was ubiquitous globally in soils and sediment and was attributed to anthropogenic sources. Perhaps the new anthropogenic marker will be glyphosate levels throughout the soil column and within bodies of water. The entire fate of glyphosate when commercially sprayed is unknown, and more research is looking into its presence in the atmosphere. This is perhaps another contributing factor to the similarity of the measurements and its detection in precipitation.

While overall the differences in glyphosate concentrations from the three locations were not significant, there are observed trends that are important to talk about. The increase in depth in the streams can be attributed to the rainfall event during the sampling period as the rainfall and corresponding runoff causes the overall stream depth to increase from its baseflow. When looking at the change in overall stream depth over the course of the sampling period, there is a slight correlation between the increase in depth and increase in glyphosate for the noncommercial limited exposure location. While this is not a statistical correlation there is a peak within the similar timeframe of the two graphs (Figure 8 and Figure 9). This may be due to the increased amount of runoff that this location was exposed to. As a location in a more suburban area there is increased runoff from the concrete surfaces of urbanized areas. The other two locations experienced runoff as there were fluctuations in depth throughout the study, however they did not exhibit a correlation like the noncommercial limited exposure location. It is important to note that during the rainfall event there was no apparent dilution of glyphosate. This again supports the hypothesis of glyphosate entering streams through runoff and rainfall. Had there been a dilution in the glyphosate during the depth increase, then it would be assumed that there are base levels in the baseflow and incoming runoff dilutes it. However, what was observed was steady levels or peak levels during the depth increase. This indicates glyphosate is being carried into the streams via runoff and/or rainfall. It would make sense to assume that an increase in total dissolved solids at the locations would lead to an increase in glyphosate concentration if it is persisting in the soils. However, what was observed in this study was in fact limited to no correlation between glyphosate and total dissolved solids, and instead there was a more obvious relationship between glyphosate and water level. This further supports the idea that glyphosate is mobile and extremely soluble in water and may readily run off into streams during a rainfall event and may not be degrading in the soils as once initially thought.

Due to the newly understood persistence of this substance, more research is being done to understand the bioremediation of glyphosate to further evaluate its environmental fate. Certain bacterial strains are observed to have better abilities to degrade glyphosate in the soils compared to the natural microbial community of the soil profile. In an experimental plot, the introduction of *Achromobacter sp.* and *Ochrobactum anthropi*, increased glyphosate degradation by 2-3-fold; within 2 weeks of existing in the soil, they decreased overall glyphosate content in the contaminated soil (Ermakova, 2010). This suggests that remediation of soils is possible and potentially the answer to preventing glyphosate from appearing in streams. The main contribution of glyphosate in the streams must be from the application of glyphosate on soils and land surfaces. The remediation of said contaminated soils as well as limiting application dosages is suggested as the means to prevent glyphosate from appearing in measurable amounts in streams.

There are many research possibilities in this field as there are still many unknowns when it comes to glyphosate fate and transport. Atmospheric glyphosate is a mostly undiscussed field and even glyphosate presence in bodies of water is not well understood. There is also little research on glyphosate's ability to leach into groundwater and the implications of it traveling that far into the ground. Bioremediation and mechanical remediation are other fields that can be further explored when it comes to this chemical. There is some research in glyphosate toxicity in the chromosomes of developing plants that could help to further explain its ability to damage DNA. While this research specifically did not find statistical significance in the main research question, analyzing results suggests interesting trends that could be further pursued in other research and suggests that there could be background exposure occurring more frequently than once thought.

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